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Revisión | Review

Mycotoxin in Medicinal/Aromatic Herbs – a Review

[Micotoxinas en hierbas medicinales y/o aromaticas - Revisión]

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Abstract
Owing to their conditions of production, and sometimes to poor storage and transportation conditions, herbal products are susceptible to fungal contamination and development. This can lead to the accumulation of mycotoxins in this kind of commodities. Consequently, herbal products can be contaminated with fungal toxins, such as aflatoxins, ochratoxins and other mycotoxins, which pose a serious risk to public health. This paper reviews the main aspects regarding mycotoxin contamination of medical/aromatic herbs, in the context of the importance of this product in a global market. Moreover, the effect of processing on final contamination of derived foods, as well as the analytical methodology commonly employed in fungal and mycotoxin analysis in this type of products was reviewed.

Keywords: aromatic herbs, medicinal herbs, mycotoxins, moulds.

Resumen
Debido a sus condiciones habituales de producción, así como a condiciones inadecuadas de almacenamiento y de transporte, los productos a base de plantas medicinales o aromáticas son susceptibles a la contaminación por mohos, lo que puede conducir a la acumulación de toxinas fúngicas tales como las aflatoxinas, ocratoxinas y otras micotoxinas, lo que supone un grave riesgo para la salud pública. Este artículo revisa los principales aspectos de la contaminación por micotoxinas de las hierbas medicinales y/o aromáticas, teniendo en cuenta el contexto de este producto en un mercado globalizado, así como el efecto del procesado sobre la contaminación final de estos productos. También se revisa la metodología más comúnmente empleada en el análisis de mohos y micotoxinas en este tipo de productos.

Palabras Clave: hierbas aromáticas, hierbas medicinales, micotoxinas, mohos.

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INTRODUCTION

Occurrence of mycotoxins like aflatoxins (AF), ochratoxin A (OTA), zearalenone (ZEA) and various trichothecenes in herbal products has been reported worldwide. Field production, as well as lack of hygiene and control during raw material storage, processing or distribution of this kind of commodities, can lead to fungal growth and subsequent mycotoxin production. This review aims to address the problem of occurrence of mycotoxigenic species and mycotoxin contamination on aromatic/medicinal herbs. Special emphasis will be placed on microbiota and mycotoxin contamination as well as on analytical methods for mycotoxins in herbs.

Herbs: Diversity and Taxonomy

Medicinal herbs contain some active principles which can have therapeutic applications. All the aromatic plants are medicinal, but not all medicinal herbs are aromatic. Aromatic herbs may be defined as the dried part of the plant such leaf, stem, root, flower, or seed used to impart flavour, odour or colour (DARP, 2002).

The part of the herb to be employed and its presentation depend on the intended use for the herb. In its natural state (fresh or dried) herbs are mainly marketed in herbal stores, but transformed into essential oils, aromas or extracts which are often used in the pharmaceutical, perfume, cosmetic, decor and food industries (DARP, 2002, Moré *et al.*, 2007).

Sometimes literature on medicinal and/or aromatic herbs is confusing because of multiple designations for the same plant depending on the final use. For a given plant it is possible to find several denominations: species name, pharmaceutical name (which changes depending on the part of plant used) and common name (which varies with country, language and usage). Thus the best alternative is to use the species name.

For the reasons mentioned above, in this article we are not going to make distinction between aromatic and medicinal herbs, so the reference to the name of the herbs will be independent of its intended use. Reviewing the overall levels of moulds and mycotoxins contamination that exists in herbs is the aim of this review.

Plants are divided into families in which related plants are grouped together based on the similarity of morphological characteristics. Most of the medicinal and aromatic plants belong to the following families (Chiej, 1983, Garland, 1989, Teuscher *et al.*, 2005):

– Compositae or Daisy family (e.g., chamomile - *Matricaria chamomile*, true chamomile - *Anthemis nobilis*, marigolds - *Calendula* spp., daisy - *Bellis annua*, wormwood - *Artemisia absinthium*, chicory - *Cichorium intybus* and *C. spinosa*, milk thistle - *Silybum marianum*, silver ragwort - *Senecio bicolour* and artichoke - *Cynara cardunculus*);
– Labiatae or Mint family (e.g., lavanders, mints, thymes, rosemary - *Rosmarinus officinalis*, horehound - *Marrubium vulgare* and balm - *Melissa officinalis*);
– Umbelliferae or Carrot family (e.g., bullwort - *Ammi majus*, wild celery - *Apium graveolens*, wild carrot - *Daucus carota*, sea holly - *Eryngium maritima*, fennel - *Foeniculum vulgaris*, anise - *Pimpinella anisum*, wild parsley - *Petroselinium crispum*, hemlock - *Conium maculatum* and alexanders - *Smyrnium olusatrum*);
– Leguminosae or Pea family (e.g., carob tree - *Ceratonia siliquia*, pea - *Pisum sativum*, white and red clovers - *Trifolium repens* and *T. pratense*, false acacia - *Robinia pseudoacacia*, Judas tree - *Cercis siliquastrum*, alfalfa - *Medicago sativa* and fenugreek - *Trigonella foenum-graecum*);
– Roseaceae or Rose family (e.g., bramble - *Rubus ulmifolius*, rose - *Rosa gallica*, wood strawberry - *Fragaria moschata*, quince - *Cydonia oblongata*, round pear - *Pyrus amydaliformis*, loquat - *Eriobotrya japonica*, hawthorn - *Crataegus monogyna*, peach, almond and apricot - *Prunus persica*, *P. amygdalus* and *P. armeniaca*);
– Rutaceae or Rue family (e.g., wall and garden rues - *Ruta chalepensis* and *R. graveolens*, rue - *R. montana*, orange - *Citrus aurantium*, bitter orange tree - *Citrus aurantium*, lemon - *Citrus limon*, tangerine - *Citrus paradise*, grapefruit - *Citrus paradisi*);
– Solanaceae or Potato family (e.g., white henbane - *Hyoscyamus albus*, mediterranean withania - *Withania somnifera*, garden thorn apple - *Datura metel*, glaucous tobacco - *Nicotiana glauca* and black nightshade - *Solanum nigrum*);
– Cruciferae or Cress family (e.g., wallflower - *Cheiranthus cheiri*, bitter cress - *Cardamine hirsuta*, shepherd's purse - *Capsella bursa-pastoris*, black mustard - *Brassica nigra*, horseradish - *Armoracia rusticana*, hedge mustard - *Sisymbrium officinale*, white mustard - *Sinapis alba*, wild radish

- *Raphanus raphanistrum*, watercress - *Nasturtium officinale*);
– Liliceae or Lily family (e.g., asphodel - *Asphodelus aestivus*, wild asparagus - *Asparagus aphyllus*, seaside squill - *Drimia maritima*, mediterranean smilax - *Smilax aspera*, greater butcher's broom - *Ruscus hypophyllum*, butcher's broom - *Ruscus aculeatus*, tassel hyacinth - *Muscari comosum*, madonna lily - *Lilium candidum*, bluebell - *Hyacinthus orientalis*, aloe - *Aloe vera*, garlic - *Allium sativum*, garden onion - *Allium cepa*, mediterranean meadow saffron - *Colchium cupani*, meadow saffron - *Colchium autunnale*);
– Caryophyllaceae or Pink family (e.g., sandwort - *Arenaria serpyllifolia*, common chickweed - *Stellaria media*, sand spurrey - *Spergularia rubra*, nail wort - *Paronychia argentea*, smooth rupture-wort - *Herniaria glabra*, viscid sandwort - *Alsine tenuifolia*);
– Boraginaceae or Borage family (e.g., borage - *Borago officinalis*, common comfrey - *Symphytum officinale*, purple alkanet - *Anchusa asurea*, yellow gromwell - *Neatostema apulum*, viper's bugloss - *Echium vulgare*, southern hound's tongue - *Cynoglosum creticum*);
– Ranunculaceae or Buttercup family (e.g., pheasant's eye - *Adonis annuus*, lesser celandine - *Ranunculus ficaria*, poppy anemone - *Anemone coronaria*, love in the mist - *Nigella damascena*, short-spurred larkspur - *Delphinium staphysagria*, larkspur - *Delphinium ajacis*, traveller's joy - *Clematis vitalba*, evergreen traveller's joy - *Clematis cirrhosa*);
– Papaveraceae or Poppy family (e.g., greater celandine - *Chelidonium majus*, opium poppy - *Papaver somniferum*, common poppy - *Papaver rhoeas*, sea poppy - *Glaucium flavum*, fumitory - *Fumaria officinalis*, fumitory - *Fumaria capreolata*);
– Malvaceae or Mallow family (e.g., common mallow - *Malva sylvestris*, hairless cotton - *Gossypium herbaceum*, hollyhock - *Althaea rosea*, marsh mallow - *Althaea officinalis*);
– Cucurbitaceae or Cucumber family (e.g., squirting cucumber - *Ecballium elaterium*, pumpkin - *Cucurbita maxima*);
– Verbenaceae or Verbena family (e.g., vervain - *Verbena officinalis*, chaste tree - *Vitex agnus-castus*, cultivated lantana - *Lantana camara*);
– Scrophulariceae or Figwort family (e.g., snapdragon - *Antirrhinum majus*);
– Phytolaccaceae (e.g., pokeweed - *Phytolacca americana*);
– Euphorbiaceae (e.g., castor bean - *Ricinus communis*).

The Market of Herbs

Nowadays, the herbs trade enjoys great prosperity worldwide; what began as old agriculture with unselective wild-harvesting of plants, moved into selective collection to extensive cultivated crops. The great prosperity worldwide is mainly due to the opening of new opportunities for manufacturers in new segments such as functional food and cosmetics (Gruenwald, 2008).

Usually, data on herbs market size are included within the general data of the spices market. The EU market is the second largest market for seasonings, spices and herbs in the world, accounting for 1.2 billion euros. Apparent consumption of spices and herbs increased from 321 thousand tonnes in 2004 to 336 thousand tonnes in 2008, indicating moderate average annual growth. The leading consuming EU markets are the UK, Germany, Romania and Hungary, together accounting for 58% (CBI, 2010).

The global herbal market is divided among Germany (28%), Asia (19%), Japan (17%), France (13%), rest of Europe (12%) and North America (11%) (Gruenwald, 2008). The EU market is the largest market for spices and herbs in the world and the most consumed herbs are thyme and oregano (CBI, 2010).

The most commonly grown herbs in the EU countries are: basil, bay leaves, celery leaves, chives, coriander, dill tips, chervil, juniper, marjoram, oregano, parsley, rosemary, sage, savoury, tarragon, thyme and water cress. Despite their low consumption, France, Italy and Greece are important producers of dried herbs (CBI, 2010).

After several years of decline, the total EU imports of herbs and spices increased and reached 988 million euros in 2007; as a result of that, the annual average growth rate was 4%. Leading EU importers of herbs are Germany, the Netherlands, the UK, France and Spain. These countries also share the trait of being the highest importers from developing countries. A large part of the EU export trade consists of spices and herbs which are imported in bulk or in crude form, which are processed and/or repacked,

and then re-exported to other EU and overseas markets. In 2007, the total re-exports of herbs and spices by the EU member countries reached 200 thousand tonnes, representing a value of 616 million euros (CBI, 2010).

On the other hand, in the United States, the herbal dietary supplement market increased in sales in 2010 in comparison to 2009, continuing a steady growth trend since 2003. In 2010, total herb sales reached a turnover of 5,200 million U.S. Dollars, representing a 3.3% increase compared to the previous year. Although the rate of growth is less than the 4.8% growth rate observed from 2008 to 2009, this increase perpetuates the trend of positive growth during 9 of the past 11 years (2000-2010 period) (Blumenthal *et al.*, 2011).

Microbiota of Herbs

In this kind of commodities production conditions may lead to an increasing risk of microbial contamination. Thus microbial spoilage of the raw herbal material is one of the factors governing the global market of herbs.

Although a large range of microorganisms can be found in herbs, including bacteria, yeasts and moulds, studies concerning microbial contamination are rare.

Some studies showed that aerobic mesophilic bacteria, enterobacteria and coliforms can be detected in herbal samples (García *et al.*, 2001; Guglielminetti *et al.*, 1996; Halt and Klapec, 2005; Kneifel *et al.*, 2002; Tournas *et al.*, 2006). It is possible to find the following bacterial genera: *Bacillus*, *Proteus*, *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Micrococcus*, *Corynebacterium*, *Streptococcus*, *Enterococcus*, *Alcaligenes*, *Citrobacter* and *Providencia* (Esimone *et al.*, 2002). Although the presence of pathogenic microorganisms is relatively rare, there are some exceptions, such as, *Bacillus cereus*, *Clostridum perfringens*, *Campylobacter jejuni*, *Escherichia coli*, *Escherichia vulneris*, *Enterobacter aerogenes*, *Enterobacer cloacae*, and *Pseudomonas aeruginosa* (Czech, 2001; Esimone *et al.*, 2002; Guglielminetti *et al.*, 1996; Leimbeck, 1987; Martins *et al.*, 2001a). Table Nº 1 presents yeast contamination in some herbal samples.

Usually, herbal raw materials are contaminated by different fungal species other than yeast, some of them being mycotoxigenic (Table Nº 2). The genus *Aspergillus* is the most frequently found in this kind of commodities (Abe *et al.*, 2008; Abeywickrama and Bean, 1991; Bugno *et al.*, 2006; Chourasia, 1995; Cventinć and Pepeljnjak, 1999; Elshafie *et al.*, 1999; Horie *et al.*, 1979; Roy *et al.*, 1988; Sahay and Prasad, 1990; Singh *et al.*, 2008).

Despite the beneficial effects of herbs, some microorganisms and their metabolites can be found which could lead to serious health problems. Among the microorganisms that may be present, moulds are the most relevant, mainly due to their mycotoxins production capacity.

Table Nº 1
Reported yeast contamination in herbal samples

Yeast specie	Herbal product	Reference
Aureobasidium spp.	Platycodon	Hitokoto *et al.*, 1978
Candida guilliermondii	Unavailable data	Martins *et al.*, 2001a
Candida spp.	Unavailable data	Halt and Klapec, 2005
Cryptococcus albidus	Chamomile, linden	Martins *et al.*, 2001a
Cryptococcus laurentii	Chamomile, corn silk	Martins *et al.*, 2001a
Kloeckera japonica	Unavailable data	Martins *et al.*, 2001a
Rhodotorula glutinis	Chamomile, linden, pennyroyal	Martins *et al.*, 2001a
Rhodotorula mucilaginosa	Chamomile, garden sage, linden	Martins *et al.*, 2001a
Rhodotorula rubra	Coriander	Guglielminetti *et al.*, 1996
Saccharomyces cerevisiae	Unavailable data	Martins *et al.*, 2001a
Saccharomyces spp.	Unavailable data	Halt and Klapec, 2006
Not identified yeasts	Ginseng supplements	Czech *et al.*, 2001; Halt and Klapec, 2006; Tournas *et al.*, 2006

Table Nº 2
Fungal contamination described in herbs.

Specie name (Common name)	Moulds[(1)]	References
Artemisia absinthium (Wormwood)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Acacia concinna (Shikakai)	*A. flavus, A. niger, A. ochraceus, Curvularia spp., F. moniliforme, M. mucedo, P. citrinum, R. stolonifer*	Roy and Kumari, 1991
Achyranthes aspera (Prickly-Chaff)	*A. flavus, A. niger, A. ochraceus, Fusarium* spp., *F. moniliforme, Penicillium* spp., *P. citrinum*	Roy and Chourasia, 1990a
Acorus calamus (Sweet Flag)	*A. flavus, A. niger, A. ochraceus, Fusarium* spp., *F. moniliforme, Penicillium* spp., *P. citrinum*	Roy and Chourasia, 1990a
Adhatoda vasica (Malabar Nut)	*Abs. corymbifera, Alt. alternata, A. flavus, A. niger, A. ochraceus, C. herbarum, Fusarium* spp., *F. moniliforme, Mucor* spp., *Penicillium* spp., *P. citrinum, Pestalotia* spp., *T. viride, Umbelopsis* spp., other moulds	Abeywickrama and Bean, 1991; Roy and Chourasia, 1990a; Singh *et al.*, 2008
Aerva lanata (Boor)	*A. flavus, A. niger, A. wentii, P. janthinellum*	Abeywickrama and Bean, 1991
Aframomum melegueta (Grains of Paradise)	*Aspergillus* spp., *Fusarium* spp., *G. candidum, Penicillium* spp., *Rhizopus* spp.	Efuntoye, 1996
Allium sativum (Garlic)	*A. niger, Circinella* spp.,*Cunninghamella* spp., *Penicillium* spp., *Rhizopus* spp.	García *et al.*, 2001
Aloysia triphylla (Lemon verbena)	*A. flavus,A. ochraceus*	Rizzo *et al.*, 2004
Alternanthera pungens (Khaki Weed)	*A. flavus, A. ochraceus, F. equiseti, F. oxysporum, F. semitectum*	Rizzo *et al.*, 2004
Althaea officinalis (Marshmallow)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Alysicarpus vaginalis (Alyce Clover)	*A. flavus, A. niger, A. wentii, P. janthinellum*, other moulds	Abeywickrama and Bean, 1991
Arctium lappa (Burdock)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Arctostaphylos uva-ursi (Bearberry)	*A. flavus*	Rizzo *et al.*, 2004
Asparagus racemosus (Asparagus)	*Aspergillus spp., A. flavus, A. niger, C. herbarum, Em. nidulans, Fusarium* spp., *Mucor* spp., *P. italicum, R. stolonifer, T. viride*, other moulds	Chourasia, 1995; Singh *et al.*, 2008
Atractylodes japonica (Japanese Atractylodes)	*A. flavus,A. glaucus, A. niger, A. versicolor, Mucor* spp., *Penicillium* spp., *Rhizopus* spp., other moulds	Hitokoto *et al.*, 1978
(Atractylodes)	*A. glaucus, A. niger, Penicillium* spp., *Rhizopus* spp.	Hitokoto *et al.*, 1978
Atropa belladonna (Belladonna)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Azadirachta indica (Neem)	*Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *M. fragilis, Penicillium* spp., *Rhizopus* spp., *T. viride*	Efuntoye, 1996
Baccharis articulata	*A. flavus, A. ochraceus*	Rizzo *et al.*, 2004

(Birch)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Blepharis edulis	*A. flavus, A. niger, A. ochraceus, Chaetomium* spp., *Curvularia* spp., *F. moniliforme, M. mucedo, R. stolonifer*	Roy and Kumari, 1991
Blepharocalyx tweediei	*A. flavus*	Rizzo *et al.*, 2004
Peumus boldus (Boldo)	*A. ochraceus, F. oxysporum*	Rizzo *et al.*, 2004
Caesalpinea digyna (Teri Pods)	*A. flavus, A. niger, A. ochraceus, Chaetomium* spp., *M. mucedo, R. stolonifer*	Roy and Kumari, 1991
Calendula officinalis (Marigold)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Camellia sinensis (Black tea)	*A. flavus, A. niger, Paecilomyces* spp., *Penicillium* spp.	Elshafie *et al.*, 1999
Capsella bursa-pastoris (Shepherd's Purse)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Carum carvi (Caraway)	*Alternaria* spp., *A. flavus, A. niger, A. ochraceus, A. terreus, Fusarium* spp., *Penicillium* spp., *Rhizoctonia* spp.	Abou-Arab *et al.*, 1999
Cardiospermum halicacabum (Heart Seed)	*A. flavus, A. niger, R. stolonifer, Trichoderma* spp., other moulds	Abeywickrama and Bean, 1991
Carum ajmoda (Cellary)	*Aspergillus* spp., *Fusarium* spp., *R. stolonifer*	Chourasia, 1995
Cassia spp. (Senna)	*A. flavus, R. nigricans*	Pepeljnjak and Cvetnić, 1998; Rizzo *et al.*, 2004
Cassia fistula (Golden Shower Tree)	*A. flavus, A. niger, A. ochraceus, F. moniliforme, P. citrinum, R. stolonifer*	Roy and Kumari, 1991
Cecropia adenopus (Ambay)	*A. flavus, A. ochraceus, F. oxysporum*	Rizzo *et al.*, 2004
Centella asiatica (Gotu kola)	*A. flavus, P. janthinellum, R. stolonifer*, other moulds	Abeywickrama and Bean, 1991
Chenopodium sp. (Paico)	*A. ochraceus, F. compactum*	Rizzo *et al.*, 2004
Cinnamomum zeylanicum (Cinnamon)	*Aspergillus* spp., *Chaetomium* spp., *Mucor* spp., *Syncephalastrum racemosum*, other moulds	Hitokoto *et al.*, 1978; Chourasia, 1995; Guglielminetti *et al.*, 1996
Citrus aurantium (Bitter Orange)	*A. flavus*	Rizzo *et al.*, 2004
Citrus sinensis (Orange tree leaves)	*A. candidus, A. flavipes, A. flavus, A. fumigatus, A. glaucus, A. niger, A. terreus, Cladosporium* spp., *Fusarium* spp., *Mucor* spp., *Paecilomyces* spp., *Penicillium* spp., other moulds	Martins *et al.*, 2001a
Clerodendrum serratum	*A. flavus, A. niger, A. ochraceus, Fusarium* spp., *F. moniliforme, Penicillium* spp., *P. citrinum*	Roy and Chourasia, 1990a
Cnicus benedictus (Blessed Thistle)	*A. flavus*	Rizzo *et al.*, 2004
Coriandrum sativum (Coriander)	*A. flavus, A. glaucus, A. niger, Cylindrocarpon lichenicola, Syncephalastrum racemosum*	Guglielminetti *et al.*, 1996; Rizzo *et al.*, 2004

(Coptis)	*Aspergillus* spp., *A. flavus, A. niger, Mucor* spp., *Penicillium* spp.	Hitokoto *et al.,* 1978
Crataegus oxyacantha (Hawthorn)	*A. flavus*	Rizzo *et al.,* 2004
Cuminum cyminum (Cumin)	*Absidia* spp., *Aspergillus* spp., *A. aureolus, A. fumigatus, A. niger, Circinella* spp., *Cunninghamella* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., *P. janthinellum, Rhizopus* spp., *R. stolonifer, Scopulariopsis* spp., *Trichoderma* spp., other moulds	Chourasia, 1995; Guglielminetti *et al.*, 1996; García *et al.,* 2001
(Curcuma)	*A. glaucus, A. ochraceus*	Guglielminetti *et al.*, 1996
Cymbopogon nardus (Citronella)	*A. glaucus, P. brevi-compactum*	Guglielminetti *et al.*, 1996
Cynara scolymus (Artichoke)	*A. ochraceus, F. oxysporum*	Rizzo *et al.,* 2004
Cynodon dactylon (Bermuda Grass)	*A. flavus, A. ochraceus, F. equiseti*	Rizzo *et al.,* 2004
Cyperus rotundus (Nut Grass)	*Alt. alternata, Aspergillus* spp., *Chaetomium* spp., *Curvularia* spp., *Fusarium* spp., *Penicillium* spp., *R. stolonifer, T. viride,* other moulds	Roy and Chourasia, 1989
Echium plantagineum (Purple Viper's Bugloss)	*A. flavus, A. ochraceus*	Rizzo *et al.,* 2004
Elettaria cardamomum (Cardamom)	*Alt. alternata, Aspergillus* spp., *Curvularia* spp., *Em. nidulans, Fusarium* spp., *Mucor* spp., *Penicillium* spp., *R. stolonifer, T. viride,* other moulds	Chourasia, 1995
Eleutherococcus senticosus (Siberian Ginseng root)	*A. versicolor, E. chevalieri, E. rubrum, Penicillium* spp., *Rhizopus* spp.	Tournas *et al.,* 2006
Elder flower	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Emblica officinalis (Indian gooseberry)	*Aspergillus* spp., *Chaetomium* spp., *Curvularia* spp., *Em. nidulans, Fusarium* spp., *R. stolonifer, T. viride,* other moulds	Chourasia, 1995
Equisetum arvense (Horsetail)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Equisetum giganteum (Giant horsetail)	*A. flavus, A. ochraceus, F. equiseti, F. verticillioides*	Rizzo *et al.,* 2004
Erythraea chilensis	*F. verticillioides*	Rizzo *et al.,* 2004
Evolvulus alsinoides (Dwarf Morning-glory)	*Abs. corymbifera, Alt. alternata, A. flavus, A. niger, C. herbarum, Curvularia* sp., *Fusarium* sp., *P. italicum, T. viride, Umbelopsis* sp.	Singh *et al.,* 2008
Fagonia cretica (Cretan prickly clover)	*Alt. alternata, Alt. phragmospora, Alt. tenuissima, A. flavus, A. niger, A. sydowii, A. tamarii, Chaetomium globosum, C. herbarum, C. sphaerospermum, Cochliobolus lunatus, F. moniliforme, F. oxysporum, Gliocladium catenulatum, P. chrysogenum, Phoma herbarum, Stachybotrys chartarum, U. atrum, U. Botrytis*	Abdel-Hafez and Naggar, 2006
Foeniculum vulgare (Fennel)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998

Gardenia jasminoidis (Gardenia fruits)	*A. flavus, A. glaucus, A. niger, A. terreus, A. ustus, Mucor* spp., *Penicillium* spp., *Rhizopus* spp., other moulds	Hitokoto *et al.*, 1978
Gentiana gilliesii	*A. ochraceus*	Rizzo *et al.*, 2004
Gentiana lutea (Yellow Gentian)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Glycyrrhiza glabra (Liquorice)	*Abs. corymbifera, Aspergillus* spp., *A. flavus, A. glaucus, A. ochraceus, A. niger, A. terreus, Cladosporium* spp., *C. herbarum, Fusarium* spp., *Penicillium* spp., *P. italicum, T. viride, Rhizopus* spp., other moulds	Hitokoto *et al.*, 1978; Roy and Chourasia, 1990b; Singh *et al.*, 2008
Gmelina arborea (Gmelina)	*Aspergillus* spp., *Botrytis cinerea, Curvularia* spp., *Drechslera* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp., other moulds	Roy and Chourasia, 1989
Graminis rhizoma (Couch Grass)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Hamamelis virginiana (Witch Hazel)	*A. flavus*	Rizzo *et al.*, 2004
Holarrhena antidysenterica (Tellicherry bark)	*Aspergillus* spp., *Chaetomium globosum, Curvularia* spp., *Mucor* spp., *Penicillium* spp., *Pestalotiopsis* spp., *R. stolonifer*, other moulds	Roy and Chourasia, 1990b
Hydnocarpus laurifolia	*Alt. alternata, A. flavus, A. niger, A. ochraceus, Chaetomium* spp., *Curvularia* spp., *F. moniliforme, M. mucedo, P. citrinum, R. stolonifer*	Roy and Kumari, 1991
Hygrophila spinosa (Hydrophilia)	*Alt. alternata, Aspergillus* spp., *Chaetomium* spp., *Fusarium* spp., *Memnoniella echinata, Pestalotiopsis* spp., Other moulds	Roy and Chourasia, 1989
Ichnocarpus frutescens (Ichnocarpus)	*Aspergillus* spp., *Botrytis cinerea, Curvularia* spp., *Fusarium* spp., *Memnoniella echinata, Penicillium* spp., *R. stolonifer, T. viride*, other moulds	Roy and Chourasia, 1990b
Jatropha curcas (Physic nut)	*Aspergillus* spp., *Penicillium* spp., *T. viride*	Efuntoye, 1996
Juniperus communis (Juniper)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Laurus nobilis (Bay leaves/ Laurel)	*Alternaria* spp., *A. fumigatus, A. niger, Aspergillus* spp., *Cladosporium* spp., *Cunninghamella* spp., *Monilia* spp., *Paecilomyces* spp., *Penicillium* spp., *Trichoderma* spp.	García *et al.*, 2001
Lepidium sativum (Garden cress)	*F. solani*	Rizzo *et al.*, 2004
Mangifera indica (Mango)	*Alt. humicola, Aspergillus spp., Fusarium* spp., *M. fragilis, Penicillium* spp., *Rhizopus* spp.	Efuntoye, 1996
Marrubium vulgaris (White Horehound)	*A. flavus, A. ochraceus, F. equiseti*	Rizzo *et al.*, 2004
Matricaria chamomilla, Chamomilla recutita (Chamomile)	*Absidia* spp., *A. candidus, A. flavipes, A. flavus, A. fumigatus, A. glaucus, A. niger, A. terreus, Cladosporium* spp., *Fusarium* spp., *F. compactum, Mucor* spp., *Paecilomyces* spp., *Penicillium* spp., *R. nigricans*, other moulds	Pepeljnjak and Cvetnić, 1998 ; Martins *et al.*, 2001, Rizzo *et al.*, 2004

Melissa officinalis (Lemon Balm)	*A. flavus, F. equiseti, F. verticillioides*	Rizzo *et al.*, 2004
Mentha piperita (Peppermint)	*Alternaria* spp., *A. flavus, A. niger, A. ochraceus, A. terreus, Fusarium* spp., *F. equiseti, Penicillium* spp., *R. nigricans, Trichoderma* spp.	Pepeljnjak and Cvetnić, 1998; Abou-Arab *et al.*, 1999; Rizzo *et al.*, 2004
Mentha pulegium *Lippia turbinata* (Pennyroyal)	*A. candidus, A. flavus, A. glaucus, A. niger, A. ochraceus, Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., other moulds	Martins *et al.*, 2001; Rizzo *et al.*, 2004
Mesua ferrea (Ironwood)	*Aspergillus* spp., *Chaetomium* spp., *Drechslera* spp., *Fusarium* spp., *Penicillium* spp., *R. stolonifer, T. viride*, other moulds	Roy and Chourasia, 1989; Chourasia, 1995
Minthostachys mollis (Ecuadorian Mint)	*A. flavus, A. ochraceus, F. compactum, F. semitectum, F. verticillioides*	Rizzo *et al.*, 2004
Morinda lucida	*Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp.	Efuntoye, 1996
Brassica campestris (Mustard)	*Alt. alternata, A. candidus, A. flavus, A. niger, A. ochraceus, Cladosporium* spp., *Colletotrichum acutatum, Curvularia lunata, Curvularia pallescens, Fusarium* spp., *F. moniliforme, F. semitectum, Helminthosporium* spp., *Memnoniella echinata, Monilia sitophila, P. citrinum, R. stolonifer*	Sahay and Prasad, 1990
Origanum vulgare (Oregano)	*Alt. alternata, Aspergillus* spp., *A. niger, A. versicolor, Chaetomium* spp., *Cunninghamella* spp., *Mucor* spp., *Nigrospora* spp., *Penicillium* spp., *Phoma* spp., *Rhizopus* spp., *Trichoderma* spp.	Guglielminetti *et al.*, 1996; García *et al.*, 2001
Paeonia japonica (Japanese peony roots)	*A. glaucus, A. nidulans, A. niger, A. versicolor, Mucor* spp., *Penicillium* spp., *Rhizopus* spp., other moulds	Hitokoto *et al.*, 1978
Panax ginseng (Chinese Ginseng)	*Aspergillus* spp., *A. niger, Cladosporium* spp., *E. chevalieri*, *Penicillium* spp., *Rhizopus* spp.	Tournas *et al.*, 2006
Panax quinquefolius (American Ginseng root)	*A. flavus, A. niger, Chaetomium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp.	Tournas *et al.*, 2006
Passiflora coerulea (Passion Flower)	*A. flavus, A. ochraceus, F. semitectum, F. subglutinans, F. verticillioides*	Rizzo *et al.*, 2004
Peganum harmala (Syrian Rue)	*Alt. alternata, Alt. tenuissima, A. flavus, A. fumigatus, A. niger, A. tamarii, C. herbarum, C. macrocarpum, C. sphaerospermum, Cochliobolus lunatus, Cochliobolus spicifer, Epicoccum purpurascens , F. moniliforme, F. oxysporum, Gliocladium catenulatum, P. chrysogenum, P. oxalicum, Phoma herbarum, Stachybotrys chartarum, Stemphylium botryosum, Trimmatostroma salicis, U. atrum, U. botrytis, U. chartarum*	Abdel-Hafez and Naggar, 2006
Petroselinum sativum (Parsley root)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Picrorhiza kurroa (Kuru)	*A. flavus, A. niger, A. ochraceus, Fusarium* spp., *F. moniliforme, Penicillium* spp., *P. citrinum*	Roy and Chourasia, 1990a
Pimpinella anisum (Anise)	*Alternaria* spp., *A. flavus, A. niger*, *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp.	Abou-Arab *et al.*, 1999

Piper betle (Betel nut)	*Alt. alternata, A. flavus, A. niger, A. ochraceus, Chaetomium* spp., *Curvularia* spp., *F. moniliforme, M. mucedo, P. citrinum, R.. stolonifer*	Roy and Kumari, 1991
Piper longum (Indian long pepper)	*Alt. alternata, Aspergillus* spp., *Chaetomium* spp., *Curvularia* spp., *Em. nidulans, Fusarium* spp., *Mucor* spp., *Penicillium* spp., *R. stolonifer, T. viride,* other moulds	Chourasia, 1995
Piper nigrum (Black pepper)	*Alt. alternata, Aspergillus* spp., *Chaetomium* spp., *Curvularia* spp., *Em. nidulans, Fusarium* spp., *Mucor* spp., *Penicillium* spp., *T. viride,* other moulds	Chourasia, 1995
(Plactycodon)	*A. flavus, A. glaucus, A. nidulans, A. niger, A. ochraceus , Cladosporium* spp., *Penicillium* spp., *Rhizopus* spp., other moulds	Hitokoto *et al.,* 1978
Plumbago zeylanica (White Leadwort)	*Abs. corymbifera, Aspergillus* spp., *A. flavus, A. niger, Penicillium* spp., *P. italicum, Rhizopodopsis* sp., *Rhizopus* spp., *T. viride, Umbelopsis* sp., *Mucor* sp.	Efuntoye, 1996; Singh *et al.,* 2008
Potentilla erecta (Tormentil root)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Rheum sp. (Rhubarb)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Ruta graveolens (Rue)	*A. flavus*	Rizzo *et al.,* 2004
Salvia officinalis (Garden sage)	*A. candidus, A. flavipes, A. fumigatus, A. glaucus, Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *R. nigricans,* other moulds	Pepeljnjak and Cvetnić, 1998; Martins *et al.,* 2001
Saraca indica (Ashoka)	*Aspergillus* spp., *Chaetomium* spp., *Penicillium* spp.	Chourasia, 1995
Scorzonera undulata	*Alt. alternata, Alt. tenuissima, A. flavus* group, *A. niger, A. sydowii, A. terreus, C. herbarum, C. macrocarpum, C. sphaerospermum, Cochliobolus lunatus, Cochliobolus spicifer, F. moniliforme, F. oxysporum, F. solani, P. chrysogenum, P. citrinum, P. funiculosum, Phoma herbarum, Stachybotrys chartarum, Stemphylium botryosum, U. atrum, U. botrytis*	Abdel-Hafez and Naggar, 2006
Scutellaria baicalensis (Skullcap roots)	*A. flavus, A. niger, A. ochraceus, A. terreus, Penicillium* spp.	Hitokoto *et al.,* 1978
Smilax campestris	*A. ochraceus*	Rizzo *et al.,* 2004
Solanum nigrum (Black Nightshade)	*Alt. alternata, Aspergillus* spp., *Botrytis cinerea, Memnoniella echinata, Mucor* spp., *Penicillium* spp., *Pestalotiopsis* spp., other moulds	Roy and Chourasia, 1989
Strychnos nux-vomica (Nux vomica)	*Alt. alternata, Aspergillus* spp.,*A. candidus, A. clavatus, A. flavus, A. luchuensis, A. nidulans, A. niger, A. ochraceus, A. sydowii, Chaetomium globosum, C. herborum, Curvularia* spp., *Fusarium* spp., *F. chlamydosporum, F. equiseti, Gliocladium penicilloides, Mucor* spp., *M. mucedo, Penicillium* spp., *P. citrinum, R. stolonifer, Stachybotrys* spp., *T. viride,* other moulds	Dutta and Roy, 1987; Roy and Chourasia, 1990b

Strychnos potatorum (Cleaning Nut Tree)	*Alt. alternata, A. candidus, A. clavatus, A. flavus, A. luchuensis, A. nidulans, A. niger, A. ochraceus, A. sydowii, C. herborum, F. equiseti, F. moniliforme, F. semitectum, Hormodendrum pallidium, M. mucedo, M. pusillus, Paecilomyces* spp., *Penicillium* spp., *P. citrinum, R. stolonifer*	Dutta and Roy, 1987
Symphytum officinale (Comfrey root)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Taxus baccata (Yew)	*Aspergillus* spp., *Curvularia* spp., *Fusarium* spp., *T. viride*	Chourasia, 1995
Terminalia chebula (Chebulic Myrobalan)	*Alt. alternata, A. flavus, A. niger, A. terreus, Curvularia* sp., *Fusarium* sp., *P. italicum, T. viride*	Singh *et al.*, 2008
Thymus vulgaris (Thyme)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Tilia spp. (Linden)	*Alternaria* spp., *A. candidus, A. flavus, A. fumigatus, A. glaucus, A. niger, A. ochraceus, A. terreus, Cladosporium* spp., *Fusarium* spp., *F. equiseti, F. verticillioides, Mucor* spp., *Penicillium* spp., *R. nigricans*	Pepeljnjak and Cvetnić, 1998; Abou-Arab *et al.*, 1999; Martins *et al.*, 2001; Rizzo *et al.*, 2004,
Tribulus terrestris (Small Caltrops)	*Aspergillus* spp., *A. flavus, A. niger, A. wentii, Fusarium* spp., *Mucor* spp., *P. janthinellum, Stachybotrys* spp., *T. viride*, other moulds	Roy and Chourasia, 1990b; Abeywickrama and Bean, 1991
Urtica dioica, Urtica urens (Nettle leaves)	*R. nigricans*	Pepeljnjak and Cvetnić, 1998
Valeriana officinalis (Valerian)	*A. flavus, A. ochraceus, F. equiseti, F. proliferatum, F. semitectum, R. nigricans*	Pepeljnjak and Cvetnić, 1998; Rizzo *et al.*, 2004
Vernonia amygdalina (Bitter Leaf)	*Aspergillus* spp., *Fusarium* spp., *G. albidum, M. fragilis, Penicillium* spp.	Efuntoye, 1996
Viscum album (Mistletoe)	*A. flavus*	Rizzo *et al.*, 2004
Xylopia aethiopica (Senegal Pepper)	*Aspergillus* spp., *C. bantianum*, *Fusarium* spp., *Rhizopus* spp.	Efuntoye, 1996
Zea mays (Corn silk)	*Absidia* spp., *A. candidus, A. flavus, A. fumigatus, A. glaucus, A. niger, A. terreus, Cladosporium* spp., *Fusarium* spp., *Mucor* spp., *Penicillium* spp.	Martins *et al.*, 2001
Zingiber officinale (Ginger)	*Aspergillus* spp., *A. flavus, A. glaucus, A. nidulans, A. niger, A. terreus, A. versicolor, Chaetomium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., *R. stolonifer, T. viride*, other moulds	Hitokoto *et al.*, 1978; Chourasia, 1995

(1) A.: *Aspergillus*, Abs.: *Absidia*, Alt.: *Alternaria*, B.: *Botrytis*, C.: *Cladosporium*, Cur.: *Curvularia*, E.: *Eurotium*, Em.: *Emericella*, F.: *Fusarium*, G.: *Geothichum*, M.: *Mucor*, P.: *Penicillium*, R.: *Rhizopus*, T.: *Trichoderma* and U.: *Ulocladium*.

Mycotoxins in Herbs

Nowadays, the increasing consumption of herbal products may lead to an increase in the intake of mycotoxins. Mycotoxin contamination in herbs has been described in several studies (Tables Nº 3 and Nº 4). Figure Nº 1 shows the chemical structures of the main mycotoxins found in this kind of commodities.

The most studied mycotoxins in herbs have been AF and OTA, followed by fumonisins (FBs). Contamination levels for these mycotoxins ranged

from not detected up to 1,420 µg/kg for AF, 2525.8 µg/kg for OTA and 700 µg/kg for FB.

Hitokoto *et al.*, (1978) were the only ones which analysed the presence of sterigmatocystin in several herbal products (cinnamon, corktree bark, gardenia fruits, ginger, grey atractylopes, Japanese atractylodes, Japanese golden-thread, liquorice, Japanese peony, mountain tea, pickly ash fruit and white leadwort), although this toxin was not detected in any of the samples.

Although there is little information about interaction between mycotoxins, and combined toxicity evaluation is quite complicated, it is expected that mycotoxins with similar mode of action would have, at least, an additive effect (Speijers and Speijers, 2004). There are only a few studies of simultaneous occurrence of mycotoxins in herbs (Hitokoto, 1978; Chourasia, 1995; Patel *et al.*, 1996; Halt, 1998; Santos *et al.*, 2009).

Although the European Union has established legal limits for AF, OTA, deoxynivalenol (DON), zearalenone (ZEA) and FBs in a wide variety of food related items, in the case of spices and herbs there is only official legislation for AF and OTA contamination in *Curcuma longa* (turmeric), *Mystica fragrans* (nutmeg) *Pipper* spp. (pepper) and *Zingiber officinale* (ginger), the limits being 10 and 30 µg/kg, respectively (EC, 2006a; EU, 2010a). The European legislation also set limits for OTA in *Glycyrriza* spp. (liquorice) and liquorice extracts, set at 20 and 80 µg/kg, respectively (EU, 2010a). On the other hand, the European Pharmacopeia establishes that AFB_1 should be tested in herbal products, but does not give any application limit (European Pharmacopeia, 2006).

Table Nº 3
Incidence of *Aspergillus* and *Penicillium* mycotoxins in herbal products[(1)].

Specie name (Common name)	AFB_1 (µg/kg)	AFB_2 (µg/kg)	AFG_1 (µg/kg)	AFG_2 (µg/kg)	AFt (µg/kg)	OTA (µg/kg)	Citrinin (µg/kg)	Reference
Acacia catechu (Cutch Tree bark)	90	-	-	-	-	-	-	Roy *et al.*, 1988
Acacia concinna (Shikakai)	80-1,130	-	-	-	-	-	10-760	Roy and Kumari, 1991
Achillea millefolium (Yarrow)	ND	-	-	-	-	ND	-	Halt, 1998
Achyranthes aspera (Prickly-Chaff)	750	-	-	-	-	-	Detected	Roy and Chourasia, 1990a
Aconitum ferox (Indian Aconite roots)	ND	-	-	-	-	-	-	Roy *et al.*, 1988
Acorus calamus (Sweet Flag)	1,100	-	-	-	-	-	Detected	Roy and Chourasia, 1990a
Adhatoda vasica (Malabar Nut)	670	-	-	-	-	-	-	Roy and Chourasia, 1990a
Aerva lanata (Boor)	500	-	-	-	-	-	-	Abeywickrama and Bean, 1991
Allium sativum (Garlic)	- ND	- ND	- ND	- ND	ND ND	ND -	- -	Patel *et al.*, 1996; Romagnoli *et al.*, 2007
Althaea officinalis (Marshmallow)	ND	-	-	-	-	ND	-	Halt, 1998
Andrographis paniculata (King of bitters)	-	-	-	-	-	740-1,390	-	Roy and Kumar, 1993
Argyreia speciosa (Elephant creeper)	90-2,810	50-300	-	-	-	-	-	Kumari *et al.*, 1989

Asparagus racemosus (Asparagus)	1,570-2,330	220-1,040	-	-	-	-	-	Kumar and Roy, 1993
Atractylodes japonica (Japanese Atractylodes)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Atractylodes lancea (Grey Atractylodes Tuber)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Arctium sp. (Burdock root)	-	-	-	-	2.6-10.3	<LD	<LD-25.8	Santos *et al.*, 2009
Betula alba (Birch)	ND	-	-	-	-	ND	-	Halt, 1998
Blepharis edulis	10-1,040	-	-	-	-	-	ND	Roy and Kumari, 1991
Brassica campestris (Mustard)	-	-	-	-	trace-1,420	-	-	Sahay and Prasad, 1990
Caesalpinea digyna (Teri Pods)	80-1,180	-	-	-	-	-	ND	Roy and Kumari, 1991
Camellia sinensis (Green tea)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Camellia sinensis (Red tea)	-	-	-	-	801.1-853.4	3.7-4.0	18.0-22.3	Santos *et al.*, 2009
Camellia sinensis (White tea)	-	-	-	-	94.2-254.0	<LD-1.1	<LD-19.7	Santos *et al.*, 2009
Carum cravi (Caraway)	-	-	-	-	ND	-	-	Abou-Arab *et al.*, 1999
Cassia fistula (Golden Shower Tree)	80-1,110	-	-	-	-	-	10-690	Roy and Kumari, 1991
	1.5-18.6	-	-	-	-	-	-	Arranz *et al.*, 2006
	ND - 255	-	-	-	-	-	-	Müller and Basedow, 2007
Cassia spp. (Senna)	-	-	-	-	15.8-434.3	0.8-3.1	<LD-68.6	Santos *et al.*, 2009
Cinnamomum zeylanicum (Cinnamon)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
	0.98	ND	ND	ND	0.98	-	-	Romagnoli *et al.*, 2007
Clerodendrum serratum	510	-	-	-	-	-	-	Roy and Chourasia, 1990a
Coptis japonica (Japanese Gold-Thread)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Coriandrum sativum (Coriander)	790	-	-	-	-	-	-	Roy *et al.*, 1988
	-	-	-	-	0.7	4.0	-	Patel *et al.*, 1996
	-	-	-	-	-	ND	-	Lino *et al.*, 2006
	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Crataegus oxyacantha (Hawthorn)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007

Cuminum cyminum (Cumin)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Curcuma sp. (Turmeric)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Cynara scolymus (Artichoke immature florets)	-	-	-	-	10.7-12.1	<LD-5.0	<LD-29.8	Santos *et al.*, 2009
(Devil's Claw)	ND-0.2	-	-	-	-	-	-	Arranz *et al.*, 2006
Diospyros embryopteris (Evergreen tree)	250	-	-	-	-	-	-	Roy *et al.*, 1988
	-	-	-	-	-	90-890	-	Roy and Kumar, 1993
Emblica officinalis (Indian gooseberry)	280-1,070	130-670	-	-	-	-	-	Kumar and Roy, 1993
	-	-	-	-	-	930-1,740	-	Roy and Kumar, 1993
Emblica ribes (False Pepper)	360	-	-	-	-	-	-	Roy *et al.*, 1988
	80-2,660	50-550	-	-	-	-	-	Kumari *et al.*, 1989
Foeniculum vulgare (Fennel)	-	-	-	-	1.2	ND	-	Patel *et al.*, 1996
	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Gardenia jasminoidis (Gardenia fruits)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Ginkgo biloba (Ginkgo leaves)	-	-	-	-	23.0-23.3	0.8-1.1	298.7-354.8	Santos *et al.*, 2009
Glycyrrhiza glabra (Liquorice)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
	590	-	-	-	-	-	-	Roy *et al.*, 1988
	170	-	-	-	-	50	-	Roy and Chourasia, 1990b
	-	-	-	-	-	ND - 216.5	-	Bresch *et al.*, 2000
	-	-	-	-	-	1.4-252.8	-	Ariño *et al.*, 2007
Hibiscus abelmoschus (Muskseed)	850	-	-	-	-	-	-	Roy *et al.*, 1988
Holarrhena antidysenterica (Tellicherry bark)	290	-	-	-	-	-	80	Roy and Chourasia, 1990b
	-	-	-	-	-	1,140-2,340	-	Roy and Kumar, 1993
Hydnocarpus laurifolia	20-650	-	-	-	-	-	10-490	Roy and Kumari, 1991
Ichnocarpus frutescens (Ichnocarpus)	510	-	-	-	-	-	-	Roy and Chourasia, 1990b
Illicium verum (Star anise)	-	-	-	-	94.8-104.2	<LD	<LD	Santos *et al.*, 2009
Laurus nobilis (Laurel)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Lippia citriodora (Lemon verbena)	-	-	-	-	31.1-37.7	<LD-1.5	29.6-79.1	Santos *et al.*, 2009

Matricaria chamomilla Anthemis sp. (Chamomile)	ND	-	-	-	-	ND	-	Halt, 1998
	-	-	-	-	ND	-	-	Abou-Arab *et al.*, 1999
	-	-	-	-	35.8-161.0	0.8-1.0	31.7-49.3	Santos *et al.*, 2009
Maytenus ilicifolia (Espineira Santa)	ND	ND	ND	ND	ND	-	-	Braga *et al.*, 2005
Melissa officinalis (Lemon Balm)	ND	-	-	-	-	ND	-	Halt, 1998
	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Mentha sp. (Spearmint)	-	-	-	-	16.6-29.7	1.1-1.4	41.0-43.3	Santos *et al.*, 2009
Mentha piperita (Peppermint)	-	-	-	-	ND	-	-	Abou-Arab *et al.*, 1999
Morus alba (Weeping Mulberry)	ND	-	-	-	-	ND	-	Halt, 1998
Mucuna pruriens (Velvetbean)	1,160	-	-	-	-	-	-	Roy *et al.*, 1988
Ocimum basilicum (Basil)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Origanum vulgare (Marjoram)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Olea europaea (Olive leaves)	-	-	-	-	58.0-77.6	<LD-1.3	<LD-14.9	Santos *et al.*, 2009
Paeonia japonica (Japanese peony roots)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Panax quinquefolium (American ginseng)	-	-	-	-	ND-16	-	-	D' Ovidio *et al.*, 2006
Papaver somniferum (Poppy seeds)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Pavonia odorata (Fragrant silky mallow)	870	-	-	-	-	-	-	Roy *et al.*, 1988
Petroselinum sativum (Parsley)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Peumus boldus (Boldus)	-	-	-	-	32.2-86.6	1.2-1.6	15.7-62.7	Santos *et al.*, 2009
Phellodendron chinense (Corktree bark)	ND	-	-	-	-	ND	-	Hitokoto et al. 1978
Picrorhiza kurroa (Kuru)	110	-	-	-	-	Detected	-	Roy and Chourasia, 1990a
Pimpinella anisum (Anise)	-	-	-	-	ND	-	-	Abou-Arab *et al.*, 1999
Piper betle (Betel nut)	20-1,000	-	-	-	-	-	10-720	Roy and Kumari, 1991
Piper longum (Indian long pepper)	220	-	-	-	-	-	-	Roy *et al.*, 1988

Piper nigrum (Black pepper)	1,200	-	-	-	-	-	-	Roy *et al.*, 1988
	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
(Plactycodon)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Plantago lanceolata (Ribgrass leaves)	-	-	-	-	12.6-16.1	0.9-1.1	<LD	Santos *et al.*, 2009
Plumbago zeylanica (White Leadwort)	1,130	-	-	-	-	-	-	Roy *et al.*, 1988
(Prickly Ash Fruit)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Psoralea corylifolia	-	-	-	-	-	70-1,100	-	Roy and Kumar, 1993
Rhamnus frangula (Frangula bark)	-	-	-	-	32.1-64.7	1.5-4.2	26.7-38.4	Santos *et al.*, 2009
Rhamnus purshiana (Cascara Sagrada)	ND	ND	ND	ND	ND	-	-	Ventura *et al.*, 2004
Rhamnus wighatii	1830	-	-	-	-	-	-	Kumar and Roy, 1993
Rheum officinalis (Rhubarb stem)					35.7-71.2	2.1-13.9	42.2-42.9	Santos *et al.*, 2009
Rosa canina (Rose hip)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Rosmarinus officinalis (Rosemary)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Salvia fructicosa, Salvia officinalis (Sage)	-	-	-	-	23.8-25.2	1.1-17.3	51.6-273.2	Santos *et al.*, 2009
	ND	ND	ND	ND	ND	-		Romagnoli *et al.*, 2007
Sambucus nigra (Elder)	ND	-	-	-	-	ND	-	Halt, 1998
Scutellaria baicalensis (Skullcap root)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
Silybum marianum (St Mary's thistle seeds)	-	-	-	-	10.9-11.5	<LD	<LD	Santos *et al.*, 2009
Solanum xanthocarpum (Indian Nightshade)	140	-	-	-	-	-	-	Roy *et al.*, 1988
Strychnos nux-vomica (Nux vomica)	670	-	-	-	-	100	-	Roy and Chourasia, 1990b
Tacca aspera	-	-	-	-	-	300-740	-	Roy and Kumar, 1993
Taraxacum officinale (Dandelion)	-	-	-	-	11.8-21.7	<LD-10.6	21.6-96.0	Santos *et al.*, 2009
Tephrosia purpurae (Purple tephrosia)	420	-	-	-	-	-	-	Kumar and Roy, 1993
	-	-	-	-	-	940-1,580	-	Roy and Kumar, 1993

Terminalia bellerica (Belliric Myrobalan)	620	-	-	-	-	-	-	Roy *et al.*, 1988
Terminalia chebula (Chebulic Myrobalan)	180	-	-	-	-	-	-	Roy *et al.*, 1988
	1,430-2,190	570-1,340	230-670	-	-	-	-	Kumar and Roy, 1993
	-	-	-	-	-	90-1,230	-	Roy and Kumar, 1993
(Thyme)	ND	ND	ND	ND	ND	-	-	Romagnoli *et al.*, 2007
Tilia grandifolia, Tilia sp. (Linden)	ND	-	-	-	-	traces	-	Halt, 1998
Tinospora cordiflia	420-860	-	-	-	-	-	-	Kumar and Roy, 1993
Tribulus terrestris (Small Caltrops)	420	-	-	-	-	-	-	Roy and Chourasia, 1990b
	-	-	-	-	-	140-1,300	-	Roy and Kumar, 1993
Valeriana officinalis (Valerian root)	-	-	-	-	6.2-15.8	0.8-1.8	<LD-20.5	Santos *et al.*, 2009
Verbena officinalis (Vervain)	-	-	-	-	48.9-104.5	<LD-1.1	<LD-31.2	Santos *et al.*, 2009
Zingiber officinale (Ginger)	ND	-	-	-	-	ND	-	Hitokoto *et al.*, 1978
	<0.4-3.6	-	-	-	-	-	-	Arranz *et al.*, 2006
	-	-	-	-	4.2-13.5	2.1-7.5	-	Patel *et al.*, 1996

(1) ND: not detected, <LD: lower than limit of detection, AF: aflatoxin, AFt: total aflatoxins, OTA: ochratoxin A

Table Nº 4
Incidence of *Fusarium* mycotoxins in herbal products(1)

Specie name (Common name)	Fumonisins (µg/kg)	ZEA (µg/kg)	Trichothecenes (µg/kg)	Reference
Achillea millefolium (Yarrow)	-	ND	-	Halt, 1998
Achyranthes aspera (Prickly-Chaff)	-	Detected	-	Roy and Chourasia, 1990a
Adhatoda vasica (Malabar Nut)	-	Detected	-	Roy and Chourasia, 1990a
Allium sativum (Garlic)	ND	ND	DON: 14, FUS X: 5, NIV: 21	Patel *et al.*, 1996
Althaea officinalis (Marshmallow)	-	ND	-	Halt, 1998
Arctium sp. (Burdock root)	<LD	<LD-10.9	DON: <LD, T2: 0.6-1.2	Santos *et al.*, 2009
Arctostaphylos uva-ursi (Bearberry leaves)			DON: 5.2	Santos *et al.*, 2009
Betula alba (Birch)	-	ND	-	Halt, 1998

Camelia sinensis (Black tea)	FB_1: 80-280, FB_2: ND	-	-	Martins *et al.*, 2001b
	FB_1: ND-traces, FB_2:ND	-	-	Omurtag and Yazicioğlu, 2004
Camelia sinensis (Green tea)	ND	-	-	Omurtag and Yazicioğlu, 2004
Camelia sinensis (Red tea)	<LD	4.5-11.2	DON: 149.1-179.9, T2: 40.6-42.8	Santos *et al.*, 2009
Camelia sinensis (White tea)	<LD	8.3-11.2	DON: <LD-60.0, T2: 34.5-42.8	Santos *et al.*, 2009
Cassia spp. (Senna)	86.7-96.7	7.1-8.4	DON: 20.5-35.2, T2: 2.4-3.0	Santos *et al.*, 2009
	FB_1: ND-<LD, FB_2: ND			Omurtag and Yazicioğlu, 2004
Citrus sinenis (Orange tree leaves)	FB_1: 350-700, FB_2: ND	-	-	Martins *et al.*, 2001b
Clerodendrum serratum	-	Detected	-	Roy and Chourasia, 1990a
Coriandrum sativum (Coriander)	ND	3.6-6.7	DON: 21	Patel *et al.*, 1996
Cynara scolymus (Artichoke immature florets)	<LD	7.2-7.8	DON: 165.0-200.2, T2: 17.8-29.8	Santos *et al.*, 2009
Foeniculum vulgare (Fennel)	ND	7.0	ND	Patel *et al.*, 1996
	ND	-	-	Omurtag and Yazicioğlu, 2004
Ginkgo biloba (Ginkgo leaves)	<LD	9.1-9.4	DON: 63.4-134.1, T2: 19.1-29.4	Santos *et al.*, 2009
Glycyrrhiza glabra (Liquorice)	ND	-	-	Omurtag and Yazicioğlu, 2004
	647	-	-	Santos *et al.*, 2009
Illicium verum (Star anise)	140.0-146.7	2.2-10.1	DON: 275.2-321.2, T2: 40.6-60.5	Santos *et al.*, 2009
Laurus nobilis (Laurel)	ND	-	-	Omurtag and Yazicioğlu, 2004
Lippia citriodora (Lemon verbena)	<LD	6.4-14.0	DON: 119.4-143.7, T2: 18.9-28.6	Santos *et al.*, 2009
Matricaria chamomilla *Anthemis* sp. (Chamomile)	-	ND	-	Halt, 1998
	FB_1: 20-70, FB_2: ND	-	-	Martins *et al.*, 2001b
	ND	-	-	Omurtag and Yazicioğlu, 2004
	<LD-90.0	7.3-12.5	DON: 123.4-191.5, T2: 3.5-8.3	Santos *et al.*, 2009
Melissa officinalis (Lemon Balm)	-	ND	-	Halt, 1998
Mentha sp. (Spearmint)	<LD	2.1-9.3	DON: 46.9-91.1, T2: 3.9-4.9	Santos *et al.*, 2009
Mentha piperita (Peppermint)	FB_1: 160, FB_2: ND			Omurtag and Yazicioğlu, 2004
Morus alba (Weeping Mulberry)	-	ND	-	Halt, 1998
Origanum vulgare (Marjoram)	FB_1: ND-<LD, FB_2: ND	-	-	Omurtag and Yazicioğlu, 2004
Olea europaea (Olive leaves)	<LD	9.3-42.7	DON: 127.2-149.9, T2: 3.0-3.5	Santos *et al.*, 2009
Peumus boldus (Boldus)	<LD	7.0-10.3	DON: 223.1-343.5, T2: 21.9-26.7	Santos *et al.*, 2009

Plantago lanceolata (Ribgrass leaves)	<LD	1.6-7.7	DON: <LD, T2: 12.3-256.9	Santos *et al.*, 2009
Rhamnus frangula (Frangula bark)	<LD	1.5-44.1	DON: <LD-60.9, T2: <LD-12.6	Santos *et al.*, 2009
Rheum officinalis (Rhubarb stem)	<LD	3.1-24.4	DON: 58.4-241.8, T2:3.0-23.0	Santos *et al.*, 2009
Rosa canina (Rose hip)	ND	-	-	Omurtag and Yazicioğlu, 2004
Salvia fructicosa	ND	-	-	Omurtag and Yazicioğlu, 2004
Salvia officinalis (Sage)	130.0-133.3	4.7-5.2	DON: 83.6-102.2, T2: 0.6-2.5	Santos *et al.*, 2009
Sambucus nigra (Elder)	-	ND	-	Halt, 1998
Sideritis libanotica (Mountain tea)	ND	-	-	Omurtag and Yazicioğlu, 2004
Silybum marianum (St Mary's thistle seeds)	<LD-236.7	1.6-3.5	DON: <LD, T2: 17.5-35.6	Santos *et al.*, 2009
Strychnos nux-vomica (Nux vomica)	-	80	-	Roy and Chourasia, 1990b
Taraxacum officinale (Dandelion)	<LD	4.2-17.0	DON: 36.0-66.5, T2: 1.9-2.7	Santos *et al.*, 2009
Tilia grandifolia	-	ND	-	Halt, 1998
Tilia sp.	FB_1: 20-200, FB_2: ND	-	-	Martins *et al.*, 2001b
(Linden)	FB_1: ND-<LD, FB_2: ND	-	-	Omurtag and Yazicioğlu, 2004
Tribulus terrestris (Small Caltrops)	-	20	-	Roy and Chourasia, 1990b
Urtica sp. (Nettle)	FB_1: <LD-1487, FB_2:ND-<LD	-	-	Omurtag and Yazicioğlu, 2004
Valeriana officinalis (Valerian root)	<LD-96.7	1.0-4.3	DON: 38.8-64.7, T2: 10.5-13.3	Santos *et al.*, 2009
Verbena officinalis (Vervain)	<LD	4.8-9.5	DON: <LD-60.0, T2: 4.7-20.4	Santos *et al.*, 2009
Zea mays	FB_1: 50-150, FB_2: ND	-	-	Martins *et al.*, 2001b
(Corn silk)	ND	-	-	Omurtag and Yazicioğlu, 2004
Zingiber officinale (Ginger)	ND	ND	DON: 9, NIV: 34, NEO: 23, T2: 18	Patel *et al.*, 1996

(1): ND: not detected, <LD: lower than limit of detection, FB: fumonisin, DON: deoxynivalenol, NIV: nivalenol, NEO: neosolaniol, T2: T-2 toxin, FUS X: fusarenone X.

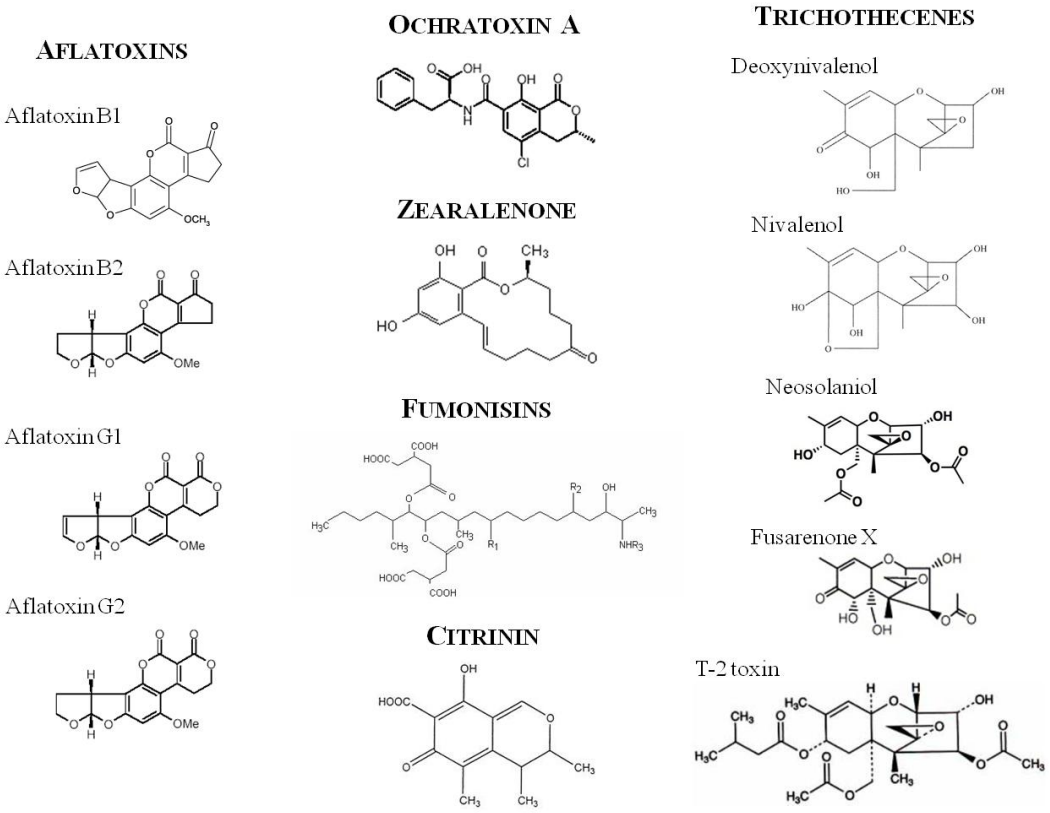
Analytical Methods for Detection of Mycotoxins in Herbs

Mycotoxin contamination in natural samples is not homogeneous and sampling schemes must be based in official regulations like the EU regulation (EC, 2006b; EU, 2010b). EU official sampling and testing methods are available only for some commodities, and might not be applicable to herbal drugs without thorough adaptation and validation (Kabelitz and Sievers, 2004; EC, 2006b).

The efficacy of the mycotoxin detection methods depends on the matrix analyzed (MacDonald and Castle, 1996; Ventura *et al.*, 2004; Goméz-Catalán *et al.*, 2005; Ip and Che, 2006). Since herbal products are complex matrices consisting of various components which can interfere with mycotoxin quantification, it is necessary to choose an extraction

and clean-up method appropriate to reduce these interferences, in order to be able to make a realistic quantification of mycotoxins (MacDonald and Caslte, 1996; Ali *et al.,* 2005; Zhang *et al.,* 2005). The extraction solvents generally used are organic solvents pure or mixed with water, or pure water, depending on the mycotoxins to be extracted. The organic pure solvents usually used are: acetonitrile, chloroform and methanol. Because herbs are complex matrices sometimes extraction is also made with addition of Tween 20 (Ali *et al.,* 2005; Zhang *et al.,* 2005; Trucksees *et al.,* 2006), phosphate buffered saline solution (Ali *et al.,* 2005; Trucksees *et al.,* 2006), salted aqueous solution (e.g., sodium bicarbonate) (Bresch *et al.,* 2000; Ali *et al.,* 2005; Ariño *et al.,* 2007) or with addition of a salt (e.g., sodium choride, potassium chloride) (Roy and Kumar, 1993; Tassaneeyakul *et al.,* 2004; Braga *et al.,* 2005; D'Ovidio *et al.,* 2006; Ip and Che, 2006; Sewran *et al.,* 2006; Trucksees *et al.,* 2006). The sample-to-extractant ratio is an important parameter because a higher ratio (more sample per solvent) results in more concentrated extracts which lead to a decreased limit of quantification (Arranz et al. 2006). For the selection of the extraction solvent it is necessary to consider the clean-up method to be used, because, for example, immunoaffinity columns (IAC) are very sensitive to some reagents (Goméz-Catalán *et al.,* 2005; Arranz *et al.,* 2006).

Figure Nº 1
Mycotoxins found in herbal products



Sometimes the problem of interfering compounds can be overcome using sophisticated clean-up methods or even a 2-step clean-up procedure (Ali *et al.,* 2005; Braga *et al.,* 2005). There are several clean-up methods, from liquid-liquid extraction (Roy and Kumar, 1993; Halt, 1998), solid phase extraction (Shank *et al.,* 1972; Patel *et al.,* 1996; Selim *et al.,* 1996; Ventura *et al.,* 2004), strong anion exchange (SAX) cartridges (Patel *et al.,* 1996; Martins *et al.,* 2001b; Omurtag and Yazicioðlu, 2004), multifunctional columns (Ali *et al.,* 2005) to IAC (Reif and Metzger, 1995; MacDonald and Castle, 1996; Patel *et al.,* 1996; Bresch *et al.,* 2000; Tassaneeyakul *et al.,* 2004; Ali *et al.,* 2005; Braga *et al.,* 2005; Goméz-Catalán *et al.,* 2005; Zhang *et al.,* 2005; Arranz *et al.,* 2006; D'Ovidio *et al.,* 2006;

European Pharmacopeia, 2006; Ip and Che, 2006; Lino *et al.,* 2006; Sewran *et al.,* 2006; Trucksees *et al.,* 2006; Ariño *et al.,* 2007; Müller and Basedow, 2007; Romagnoli *et al.,* 2007). The most selective procedures are multifunctional columns and IAC. The efficacy of IAC clean-up for mycotoxins depends on the analyzed matrix (MacDonald and Castle, 1996; Ventura *et al.,* 2004).

Many analytical methods have been developed for mycotoxins in herbs which include thin layer chromatography (TLC) (Shank *et al.,* 1972; Roy *et al.,* 1988; Kumari *et al.,* 1989; Roy and Chourasia, 1989; Roy and Chourasia, 1990a; Roy and Chourasia, 1990b; Abeywickrama and Bean, 1991; Roy and Kumari, 1991; Kumar and Roy, 1993; Roy and Kumar, 1993; Halt, 1998; Fuat *et al.,* 2006), ELISA or other immunoaffinity-based methods, HPLC with fluorescence detection (Reif and Metzger, 1995; MacDonald and Castle, 1996; Patel *et al.,* 1996; Selim *et al.,* 1996; Martins *et al.,* 2001b; Omurtag and Yazicioðlu, 2004; Tassaneeyakul *et al.,* 2004; Ali *et al.,* 2005; Braga *et al.,* 2005; Goméz-Catalán *et al.,* 2005; Zhang *et al.,* 2005; Arranz *et al.,* 2006; D'Ovidio *et al.,* 2006; European Pharmacopeia, 2006; Ip and Che, 2006; Lino *et al.,* 2006; Sewran *et al.,* 2006; Trucksees *et al.,* 2006; Ariño *et al.,* 2007; Müller and Basedow, 2007; Romagnoli *et al.,* 2007), GC/MS (Patel *et al.,* 1996) or HPLC/MS (Bresch *et al.,* 2000; Ventura *et al.,* 2004).

For some mycotoxins like AFB_1, AFG_1, FBs and trichothecenes the sensitivity of the detection method can be improved including a sample derivatization step. Generally a pre-column derivatization with *o*-phtaldialdehyde solution is used for FBs (Patel *et al.,* 1996; Martins *et al.,* 2001b; Omurtag and Yazicioðlu, 2004; Sewran *et al.,* 2006). For trichothecenes, a pre-column derivatization using Trisil TBT is used (Patel *et al.,* 1996). Nowadays the best method for AF derivatization is post-column one, which can be made using pyridinium bromide perbromide (PBPB) (MacDonald and Castle, 1996; Patel *et al.,* 1996; Zhang *et al.,* 2005; Arranz *et al.,* 2006; European Pharmacopeia, 2006; Ip and Che, 2006; Sewran *et al.,* 2006), or preferably using a KOBRA cell (Reif and Metzger, 1995; Tassaneeyakul *et al.,* 2004; Goméz-Catalán *et al.,* 2005; Arranz *et al.,* 2006; D'Ovidio *et al.,* 2006; European Pharmacopeia, 2006; Müller and Basedow, 2007; Romagnoli *et al.,* 2007) or a photochemical reactor for enhanced detection (Ali *et al.,* 2005; Arranz *et al.,* 2006; European Pharmacopeia, 2006; Trucksees *et al.,* 2006). The use of a pre-column derivatization with trifluoroacetic acid (TFA) (Ali *et al.,* 2005; D'Ovidio *et al.,* 2006; Trucksees *et al.,* 2006) or a post-derivatization using PBPB or KOBRA cell can be useful, but presents some problems related with the reagents purity, by-products formation, completeness of the reaction and stability of the reagent and products formed (Braga *et al.,* 2005). The AF derivatization with TFA can be also used to confirm the presence of AF in TLC determinations (Shank *et al.,* 1972; Roy *et al.,* 1988; Roy and Chourasia, 1989; Roy and Chourasia, 1990a; Roy and Chourasia, 1990b; Abeywickrama and Bean, 1991; Roy and Kumari, 1991; Kumar and Roy, 1993).

It should be borne in mind that there is not a universal method of analysis for each herb or herbal groups. For each analyzed herb a prior validation of the method should be made, to be sure that the mycotoxins under study are correctly quantified. Furthermore, depending on the analyzed matrix it is possible to have different interfering compounds which can elute at the same time as the mycotoxin of interest.

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